INFLUENCE OF ETHYLENE ON THE BIOCHEMICAL AND BREADMAKING PROPERTIES OF FRESHLY HARVESTED WHEAT

by

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TABLE OF CONTENTS

INTRODUCTION

The after-ripening process may be defined as the physiological and chemical changes which take place in a seed during the period between field cutting or harvesting of the grain and dormancy. It is in this post-harvest ripening period that the so-called "sweat" of wheat probably occurs. Before the introduction of the combine-harvester, the afterripening process probably occurred to a great extent in the wheat shock and therefore few difficulties were experienced with wheat harvested by the older methods. Atkinson and Jahnke (3) and Swanson (30) found, however, that freshly harvested wheat, as obtained from the combine-harvester, gave low germination values. McCalla and Newton (23) found that immature wheat samples were difficult to mill and flour yields increased with the development of the wheat kernel. Swanson (29) reported that combined wheat is likely to be unsuitable for breadmaking until it has been aged for some weeks.

As for the so-called "sweat" of wheat, no clear-cut definition of the process can be found in the scientific literature. An increase in surface moisture or dampness of freshly harvested wheat after a few days of storage has been observed by farmers, elevator operators and millers. They refer to this phenomenon as the "sweat", and it is claimed that the wheat must go through this process before it will mill properly and produce

flour which gives good baking results. No experimental evidence seems to exist on this point, except for the related phenomenon of post-harvest dormancy. Swanson (30) refers to the process as follows:

> What happens in the usual aging process of wheat is not so well understood, but it is apparent that when sweating does not take place, the rate of living continues to be very slow and the improvement associated with "aging" comes at a much slower rate.

The afterripening process has been defined otherwise by the botanist. Meyer and Anderson (24), in their standard text on botany, have defined the process as follows:

> After-ripening involves principally a series of changes in the physiological condition of the embryo which gradually converts a dormant embryo into one which can resume growth.

Miller (26), plant physiologist, has defined it as follows:

> The term "afterripening" refers to the series of chemical or physical changes, occurring within the plant or plant parts, which bring to a close the dormant period and make growth again possible.

From the above discussion, it may be inferred that the terms "post-harvest dormancy", "afterripening" and "sweat" are synonymous but that a clearer definition of all would be desirable.

Among the biochemical studies on these phenomena may be noted the work of Eckerson (13) who found that the afterripening period of several species of Crotaegus could be greatly shortened by treating the embryo with dilute acids such as hydrochloric, butyric, and acetic. She found the initial change in this period to be an increased acidity, correlated with an

increase in water-holding power, and an increase in the activity of catalase and peroxidase. In 1917 Bach, Oparin and Vener (4) reported that with the ripening of grain, enzyme activity greatly increases, reaching a maximum and beginning to fall to an insignificantly low level in the ripe seed. Kretovich (19) also found this to be true, reporting that in the later stages catalase and peroxidase decrease comparatively little and that amylase and protease almost disappeared in the process of ripening of grains. Bach, Oparin and Vener (4) found in the later stages of ripening a rather significant variation in enzyme activity. They explained this as due to weather changes, which have a definite influence on enzyme activity in the ripening grain. In the opinion of these investigators, during the ripening of grain two processes are going on: the formation of enzymes and their transformation into non-active forms termed zymogens. It is evident from the work of Proskuryakov, Bundel, and Bukharina (28) that there is a gradual weakening of the hydrolytic effect of protease with the ripening of grain.

During the later stages of kernel development, Mangels and Stoa (21) and Malloch (20) found a tendency for the diastatic activity of flour to decrease with advancing maturity of the grain from which it was milled.

Many investigators have reported that the process of grain ripening may be characterized by a continuous increase in the content of dry matter, terminating at the time of complete

ripeness. It is generally agreed by a number of authors including McCalla and Newton (22), Brenchley, Winnifred and Hall (8), Johnson and Whitcomb (17) and Thatcher (33) that the percentage of ash (mineral matter) in wheat kernels decreases as the grain develops. After the wheat was mature, McCalla and Newton (22) found that the ash content remained relatively constant.

Teller (32) found that the percentage of protein in the wheat grain decreased from its formative stage until ripeness. Brenchley (7) and Brenchley, Winnifred and Hall (8) found in the formation of the wheat grain that the percentage of protein fell rapidly at first and then became practically constant. Nonprotein nitrogen was reported by Woodman and Engledow (34) to decrease in the maturing wheat kernel.

The effect of ethylene on the afterripening process was first discovered when kerosene stoves were used to ripen fruits artificially. It was thought that the heat from the stoves ripened the fruit. It was shown by Denny (12) in 1924 that the ripening effect was due to the ethylene given off from the stove. He found a rapid appearance of yellow color in the skin of mature green lemons when exposed to ethylene diluted as much as one part per million. Chace and Church (9) found a definite increase in the ripe color of oranges, lemons and persimmons in the ethylene-treated over the untreated. In 1928, Chace and Sorber (10) demonstrated that starch is converted more rapidly to sugar in pears which undergo typical ripening when treated with ethylene. Gane (14) strengthened the view that ethylene

produces a natural rather than an artificial ripening by the observation that apples, pears, peas and bananas give off ethylene during the natural ripening process, which in turn acts on any neighboring green fruit. Chace and Sorber (11) found that ethylene had similar effects on walnuts.

Very little work has been done on freshly-harvested, ethylene-treated wheat up to this time. In 1939 experiments were conducted by Balls and Hale (5) on ethylene-treated wheat with the thought that ethylene might hasten the afterripening of combined wheat. They have shown that the bread made of the flour from the ethylene-treated wheat was of excellent texture, color and apparently normal in all respects. The bread from the untreated wheat had smaller volume, was soggy, and was decidedly green in color.

Balls and Hale (5) also carried out oxygen-absorption measurements in a Warburg apparatus which showed no difference between the rates of oxygen consumption by one gram of treated as compared with one gram of untreated wheat. They found a marked increase in the germination of the treated over the untreated wheat but it appeared to them that too much ethylene was decidedly harmful in this connection.

In 1941 Hale, Schwimmer and Bayfield (18) investigated the keeping quality of high-moisture wheat when treated with ethylene. They stored about 700 bushels each of freshly harvested wheat at 17.2 per cent moisture in two cylindrical metal bins. The wheat in one bin was treated with ethylene gas mixed in the proportion

of approximately 10,000 parts of air to one of ethylene. Temperature readings of the wheat were made at regular intervals and it was found that the untreated wheat heated considerably more than the treated. An immediate increase in the carbon dioxide output of the grain was one effect of the ethylene treatment. Germination tests showed that 80 per cent of the treated wheat and 28 per cent of the untreated wheat germinated after 18 days of storage. It is evident that the ethylene-treated samples produced better bread than those receiving no treatment during several months of storage.

No application has been made to date of the encouraging experimental findings of Balls and Hale (5) and Hale, Schwimmer and Bayfield (16) on the use of ethylene to improve the post-harvest storage, milling and baking properties of wheat. It was felt that if these results could be confirmed and shown to be of practical utility, a cheap and easily applied means would be available to accelerate the improvement of freshly-harvested wheat which otherwise is obtained by a period of storage.

With this in mind a comprehensive program of harvesting wheat at various stages of maturity was undertaken with the application of ethylene at various stages in the maturation process. Studies on the samples included determinations of moisture, ash, protein, nonprotein nitrogen, fat acidity, thiamine, catalase activity, protease activity, peroxidase activity, germination, flour pigments, maltose value, gassing power, farinograms and baking tests.

MATERIALS AND METHODS

Harvesting of Wheat

Pawnee wheat harvested with a small combine at the College Agronomy Farm was used in these studies. The first sample was harvested at 2:45 p.m., June 19, 1948; this sample contained 38.6 per cent moisture. The second sample was harvested at 2:30 p.m., June 24, 1948 and contained 19.8 per cent moisture; and the third sample was harvested at 1:50 p.m., June 30, 1948 and contained 12.3 per cent moisture. These samples were run directly into large air-tight cans in the field and were taken immediately into the laboratory for testing. Drying was done in a forced-draft tunnel drier at a temperature of 95° F.

Relationship Between Weather Factors and Moisture Content of Wheat

Small samples of wheat were harvested by hand each day to determine the moisture content of the maturing wheat. Ten to 12 wheat heads were taken, placed in an air-tight glass jar and moisture determinations were made on the whole kernel. When each sample was collected the time was noted and the air temperature was recorded, as were also the weather conditions. These data are presented in Table 1.

Table 1. Relationship between weather factors and moisture content of maturing Pawnee wheat.

Date	Maximum temperature °F.	Precipitation over 24 hours: inches	Moisture of sample per cent	Time of harvest	Temperature at harvest °F.	Weather at harvest
June 10	95	0	55.8	1:20 pm	94.2	Fair
June 11	93	0	48.6	1:15 pm	92	Partly cloudy
June 12	91	Trace	45.7	12:55 am	87	Partly cloudy
June 13	80	0	-	-	-	-
June 14	96	0	42.3	1:15 pm	92	Fair
June 15	85	2.41	37.9	1:18 pm	80	Partly cloudy
June 16	79	0	38.1	1:33 pm	77	Cloudy
June 17	78	0.17	38.8	1:25 pm	72	Cloudy
June 18	78	0.10	40.1	10:15 am	73	Cloudy
June 19	71	0	34.4	10:30 am	61	Cloudy
June 20	78	0.92	-	-	-	-
June 21	78	1.02	35.8	1:15 pm	76	Cloudy
June 22	85	0.03	32.2	1:12 pm	84	Cloudy
June 23	81	3.14	28.1	1:10 pm	78	Partly cloudy
June 24	85	0	17.2	10:30 am	80	Fair
June 25	85	0.28	21.3	1:30 pm	83	Fair
June 26	85	0.01	10.5	1:15 pm	85	Fair
June 27	82	0.45	-	-	-	-
June 28	80	3.02	26.7	1:30 pm	76	Light rain
June 29	87	0.07	10.9	1:22 pm	86	Partly cloudy
June 30	84	0	-	-	-	-

Ethylene Treatment

The wheat was treated in a 1:1,000 ethylene atmosphere in a manner similar to that used in the experiments of Balls and Hale (5). The wheat to be treated was placed in an air-tight can, having a volume of about 57 liters. The ethylene was collected by water displacement in a 57 ml test tube. The test tube of ethylene was inserted into the wheat, open end down, and after the can was sealed it was rolled over and over to insure contact of the ethylene gas with all of the wheat. This ethylene-treated wheat was then set aside for 24 hours at room temperature.

Designation of Samples

The following simple code letters were used to designate the samples, due to the complexity of the experimental design and the number of treatments of each wheat sample.

A - Wheat harvested at 38.6 per cent moisture

B - Wheat harvested at 19.8 per cent moisture

C - Wheat harvested at 12.3 per cent moisture

D - Sample dried to about 12 per cent moisture at 95° F.

E - Sample treated with ethylene, 1:1,000 for 24 hours

R - Sample rewetted from 12 per cent to 18 per cent moisture

Using these letters in proper sequence completely describes the

history and treatment of the samples.

Experimental Materials Prepared

1. AD: Forty pounds of sample A (38.6 per cent moisture at harvest) were dried in a forced-draft tunnel drier for 24 hours at 95° F. to a moisture of 13.7 per cent.

2. AED: Sixty pounds of sample A were treated with ethylene for 24 hours, then dried for 24 hours to 14.7 per cent moisture.

3. BD: Eighty-five pounds of sample B (19.8 per cent moisture at harvest) were dried for 24 hours to a moisture of 12.7 per cent.

4. BED: Eighty-five pounds of sample B were treated with ethylene, then dried for 23 hours to 12.3 per cent moisture.

5. BDRD: Thirty pounds of sample BD were rewetted to 18 per cent moisture in a can over night and 15 pounds of this rewetted wheat were dried for 20 hours to a moisture of 11.0 per cent.

6. BDRED: Fifteen pounds of the rewetted BD sample were treated with ethylene and dried for six hours to a moisture of 12.9 per cent.

7. C: Sample C (harvested at 12.3 per cent) was not dried.

8. CE: Eighty-five pounds of sample C were treated with ethylene.

9. CRD: Thirty pounds of sample C were rewetted to 18 per cent moisture and 15 pounds of this wheat were dried for four and one-half hours to 13.2 per cent moisture.

10. CRED: Fifteen pounds of the rewetted C sample were treated with ethylene, then dried for five hours to a moisture of 12.9 per cent.

The samples were rewetted and treated with ethylene to determine the utility of ethylene treatment on tempered wheat. Swanson (31) found that the rewetting and drying of freshly-harvested wheat apparently caused an improvement in properties of the flours produced from these wheats.

Experimental Studies on Samples

All determinations on the wheat were made within five days after the date of harvest. These were as follows:

1. Moisture, ash, protein, and nonprotein nitrogen
2. Fat acidity, thiamine
3. Catalase, protease and peroxidase activity
4. Germination

The following determinations were made on the flour milled from the wheat samples.

1. Moisture, protein, ash and pigments
2. Maltose value, gassing power
3. Farinograms, absorption and valorimeter values
4. Baking tests

Methods of Analysis

Moisture. Since it was planned to harvest the samples at 30, 18 and 12 per cent moisture, determinations of moisture were made on samples that were hand picked in the field each day as was indicated in Table 1. The two-stage moisture procedure (Cereal Laboratory Methods, 1) was used on samples A and B. All other samples were ground in an intermediate Wiley laboratory mill, using a 40-mesh sieve. The moisture determinations were made by the one-stage procedure (Cereal Laboratory Methods, 1).

Other Determinations. The following seven determinations were carried out precisely as directed in Cereal Laboratory Methods (1):

1. Ash
2. Protein
3. Thiamine
4. Fat acidity
5. Maltose value
6. Gassing power
7. Flour pigments

Nonprotein Nitrogen. The percentage of nonprotein nitrogen was determined by the method of Becker, Milner and Nagel (6). This method consisted of precipitating the protein with trichloroacetic acid, filtering, and analyzing the filtrate for total nitrogen.

Germination. Germination tests were performed by the Kansas State Seed Laboratory, Manhattan, Kansas.

Baking Tests. Bread baking tests were carried out according to the standard A.A.C.C. baking test procedure, using an enriched formula as follows:

	Per cent
Flour	100
Sugar	6
Salt	1.5
Malt extract	0.25
Milk solids	4
Shortening	3
$KBrO_3$	0, 1, 2 and 3 mg
Water	As needed

Farinograms. Farinograms were made according to the procedure specified by the Brabender Corporation to determine the absorption and valorimeter values, an arbitrary numerical value of flour quality. The small mixing bowl was used employing 50 g of flour (14 per cent moisture basis) with the thermostat maintained at 30° C.

Proteolytic Activity. Proteolytic activity was determined by a modified Ayre-Anderson procedure as standardized by Miller (25). This method involves the hydrolysis of a hemoglobin substrate by the enzyme in a mixture at controlled temperature and pH. After the hydrolysis period the undigested protein is precipitated with trichloroacetic acid, the mixture is filtered,

and aliquots of the filtrate are analyzed for total nitrogen by the standard Kjeldahl-Gunning-Arnold procedure. The proteolytic activity is expressed as mg nitrogen per 10 g of flour.

Catalase Activity. Catalase activity was determined by the titrimetric method as standardized for vegetables by the method given in Methods of Analysis of the A.O.A.C. (2). This procedure involved grinding a 50 g sample of wheat in a Waring blendor for three minutes with one g of calcium carbonate and sufficient water to make a total volume of 200 ml. This mixture was then centrifuged and the clear solution poured off. To one ml of the extract, water was added to give a volume of 43 ml and five ml of a buffered dextrose solution. This mixture was mixed, kept at 0° C. and two ml of 0.1 N H_2O_2 added. Immediately after addition of the H_2O_2, it was thoroughly mixed, a "zero-time" aliquot was removed, and run into a H_2SO_4 molybdate solution. Ten ml aliquots were also removed at 5, 10, and 15 minutes. To this mixture was added five ml of $Na_2S_2O_3$-KI solution, mixed, and the excess $Na_2S_2O_3$ titrated with a standard iodine solution. A blank determination was made in exactly the same manner as just described except water was added in place of H_2O_2.

The catalase activity or content of the samples is expressed in terms of "Katalase Fahigkeit":

$$K_r = \frac{\frac{1}{t_a - t_b} \log \frac{\text{titer No. } H_2O_2 - \text{titer at } t_a}{\text{titer No. } H_2O_2 - \text{titer at } t_b}}{\text{g of sample per 50 ml reaction mixture}}$$

Peroxidase Activity. Peroxidase activity was determined by the Morris and Lineweaver (27) method as adapted for wheat flour Kaslow (18). The determination was carried out as follows:

Four g of ground wheat were weighed into a 125 ml Erlenmeyer flask and 44 ml of 0.1 M ammonium acetate as a buffer solution were added. The flask was then stoppered and shaken for 15 minutes. The extraction slurry was centrifuged and the resultant supernatant liquid was ready for the color test. To each of two test tubes containing nine ml of buffer, one ml of extract was added, followed by 0.20 ml of a 10 per cent solution of guaiacol in absolute ethyl alcohol. The tubes were shaken thoroughly and to one of the pair one drop of three per cent H_2O_2 was added, then shaken and the time noted. Both tubes were immediately placed in the dark for exactly 30 minutes. At the end of this period, each solution was diluted 1:30 with distilled water and the transmittance of the peroxide-treated solution was measured with an Evelyn Photoelectric colorimeter, using the untreated solution as the reference. The optical density of the test solution, obtained from the measurement of per cent transmittance, was multiplied by the dilution factor of the original extract, by the dilution of the color test, and finally by 100. The resulting number represents the peroxidase activity of the original extract.

EXPERIMENTAL

Results of Preliminary Studies

Preliminary studies on the influence of ethylene on Pawnee wheat of the 1947 crop were conducted to perfect techniques and become better acquainted with each method. This was necessary because a great deal of work had to be accomplished in a very short time after samples of fresh grain were harvested. The preliminary studies were concentrated on enzyme determinations and baking tests. The wheat was treated with ethylene at two moistures; the moisture at which it was obtained from the bin (12.6 per cent) and after conditioning it to 16 per cent moisture.

Proteolytic activity was determined in duplicate on three occasions on ethylene treated and untreated wheat samples allowing about seven days to elapse between each sample examined. The average proteolytic activity of the untreated sample was 35.96 mg nitrogen per 10 g sample and the treated sample was 35.75. This showed that there was no effect of the ethylene treatment on the proteolytic activity of these wheat samples.

The catalase activity of shorts, malted wheat flour, and ethylene-treated and untreated wheat was determined. These materials were chosen because it was expected that they might show a wide range of catalase activity. The weight of sample extracted and volume of extract used in the reaction mixture

depended on the catalase activity of the sample and these were determined by a method of trial and error. In the first two determinations on shorts a 50 g sample was extracted and 10 ml of the extract used. The activity proved to be too high for the amount of hydrogen peroxide present, since the hydrogen peroxide was totally decomposed before five minutes had elapsed. Extracting 40 g of shorts and using 2 ml of the extract gave better results. The average K_f value of this particular sample of shorts was found to be 0.1352. Knowing the proper proportions to use with shorts, it was possible to estimate the proportions needed in the determinations on malted wheat flour and wheat. Extracting 20 g of malted wheat flour and using 5 ml of the extract gave good results and an average K_f value of 0.0974 was obtained. Forty grams each of the ethylene treated and untreated wheat samples were extracted and 5 ml each of the extracts used. This resulted in an average K_f value of 0.0704 on the untreated sample and 0.0679 on the treated sample. There seemed to be no effect of ethylene on the catalase activity of the wheat samples.

In the determination of peroxidase activity, it was necessary to determine a dilution of the color test suitable for reading in the colorimeter. A total of 19 determinations was made, varying the dilution from 1:20 to 1:40. These determinations were made on the untreated wheat only and showed a variation in peroxidase activity from 18,100 to 25,000. The most consistent results were obtained using a 1:30 dilution, there-

fore it was decided to use this dilution in the determinations on the freshly harvested wheat samples.

Three duplicate bakes were conducted with the flour milled from the 1947 wheat. Samples No. 1 and 2 were treated with ethylene before conditioning and No. 3 after conditioning the sample to 16 per cent moisture for milling. The baking results are given in Table 2. The ethylene treatment did not appear to affect the results in any way.

Results of Experiments with Fresh Grain

The moisture, ash, protein, and nonprotein nitrogen determinations were made in duplicate and thiamine and fat acidity made in triplicate. The average results are given in Table 3. As was to be expected, there was a slight decrease in the percentage of ash with an increased maturity of the sample, but there was no evident effect of the ethylene treatment. There were no significant differences in the moisture, protein, or thiamine content of the dried sample with increased maturity, or with ethylene treatment. There seemed to be a general increase in the nonprotein nitrogen content of the treated sample over the untreated. There was a definite decrease in fat acidity with increasing grain maturity. There was an indication that ethylene lowered the fat acidity of all samples except sample C.

Table 2. Effect of ethylene on the breadmaking quality of flour milled from wheat of the 1947 crop.

Sample	Loaf volume Mg $KBrO_3$ 0	2	4	6	Per cent absorption	Mixing time min.	Crumb grain Mg $KBrO_3$ 0	2	4	6
#1 Untreated	632	815	805	807	62.3	2	85	92	94	93
#1 Treated	652	785	780	750	62.3	2	85	92	94	93
#2 Untreated	622	785	785	782	63.3	2	84	92	95	94
#2 Treated	620	787	780	767	63.3	2	85	91	94	93
#3 Untreated	610	742	772	762	62.5	2	84	91	94	94
#3 Treated	600	755	775	767	62.5	2	83	91	94	94

Table 3. Effect of ethylene on chemical properties of wheat.

Sample	Per cent moisture	Dry matter basis: Per cent ash	Per cent protein	mg nitrogen per 1 g wheat	Mg thiamine per lb.	Fat acidity[1]
				Nonprotein nitrogen		
AD	13.7	1.65	12.17	1.9	3.01	7.7
AED	14.7	1.62	12.25	2.1	2.26	7.3
BD	12.7	1.60	12.85	0.5	2.08	6.8
BED	12.3	1.60	12.53	1.7	2.89	6.1
BDRD	11.0	1.61	12.75	0.6	4.48	6.0
BDRED	12.9	1.57	12.34	1.2	3.69	5.5
C	12.3	1.57	12.19	1.0	3.13	5.5
CE	12.7	1.60	13.17	1.5	2.86	7.6
CRD	13.2	1.65	12.37	1.5	2.87	6.3
CRED	12.9	1.59	12.29	1.4	3.18	6.1

[1] Fat acidity is reported as mg KOH required to neutralize the free fatty acids from 100 g of wheat on a dry matter basis.

Biochemical and Biological Determinations

Germination was found to increase with ethylene treatment. Thus a marked gain in germination in sample BED over BD and CE over C appeared. In the rewetted samples there was a decrease in germination from sample BDED to BDRED and CRD to CRED, as is shown in Table 4.

The determination of proteolytic activity was made using triplicate samples in the hydrolysis step and the total nitrogen determination made in duplicate on each triplicate sample. An average of four of the closest results is given in Table 4. Ethylene treatment of the sample harvested at 38 per cent moisture (AED) resulted in a marked decrease in proteolytic activity in comparison with the similar sample not treated with the gas (AD). The ethylene-treated samples harvested at 20 per cent moisture (BE and BED) and 12 per cent moisture (CE and CRED) showed a definite increase in proteolytic activity over the untreated samples. The proteolytic activity seemed to decrease slightly with an increase in maturity of the grain.

Catalase activity was determined by making duplicate determinations on a single extract. The K_t values were calculated for the periods: 0 to 5 minutes, 5 to 10 minutes, and 10 to 15 minutes, and an average taken, which represented the catalase activity of the sample. The only significant differences found were in the case of samples A and BD, in which the

Table 4. Effect of ethylene on germination and enzyme activity of wheat.

Sample	Dry matter basis			Germination	
	Proteolytic activity mg nitrogen/10 g wheat	Catalase activity K_f	Peroxidase activity no.	Regular method per cent	prechilled per cent
A	63.68	0.2890	32100	-	-
AE	63.13	0.1541	30700	-	-
AD	64.22	0.1432	29200	52	55
AED	61.44	0.1594	17200	54	61
B	57.85	0.1118	24300	-	-
BE	63.71	0.0988	19300	-	-
BD	63.49	0.1526	23100	44	98
BED	67.64	0.0919	15900	60	92
BDRD	63.64	0.1248	14600	65	95
BDRED	62.39	-	19800	47	97
C	55.10	0.1282	21300	83	95
CE	60.33	0.1112	21200	91	94
CRD	63.06	0.1069	21700	88	93
CRED	70.00	0.0969	18700	80	96

ethylene seemed to decrease the activity. A significant difference was considered to be a difference of at least 0.04 in the K_p values, since the results from replicate determinations were within this figure. These data are presented in Table 4.

Peroxidase activity was determined by making triplicate determinations on a single extract. In view of the work done on this determination in the preliminary studies, a significant difference will be considered to be a difference of at least 7000 in the peroxidase activity. The peroxidase activity of samples A and B was decreased by the ethylene treatment, as indicated in Table 4. There was a general decrease in the peroxidase activity with the increase in maturity of the sample.

Milling Quality

Flour-making quality of the wheat after the various treatments was evaluated using the Allis experimental mill, milling to a 95 per cent long patent. The per cent flour extraction with sample AED was lower than that with sample AD. All the other samples milled to about the same extraction. The per cent flour extraction for each sample is given in Table 5. Sample AED was also harsh on the breaks and the sizings did not seem to reduce properly. The first five wheat samples were milled on June 9, 1948, and the remaining five on June 12, 1948. The ethylene treatment did not seem to increase the ease of milling of any of the samples.

Flour Characteristics

The testing of the flour was begun the day after the milling was completed. The moisture, protein, ash, and pigment determinations were all made in duplicate, of which the average results are given in Table 5. Ethylene did not seem to affect the flour pigments but with an increase in maturity of the sample there was a slight decrease in the amount of pigments.

The determination of maltose value was made in duplicate, of which the average results, expressed as mg maltose per 10 g of flour, are given in Table 6. The ethylene treatment increased the maltose value of sample AD and CRD and lowered it in samples BD and BDRD. Valorimeter readings obtained from the farinograms did not seem to be affected by the ethylene treatment but did increase with an increase in maturity of the sample.

The gassing power determinations were made in duplicate for a period of 6 hours, taking readings about every 15 minutes the first 4 hours and every 30 minutes the remaining 2 hours. An average of the six-hour readings is given in Table 6. The ethylene treatment apparently increased the gassing power of samples AD and CRD and lowered it in samples BD and BDRD. These differences may have been due to the differences in starch susceptibility produced by the milling. Evidence supporting this possibility is the variance in per cent flour extraction and flour ash.

Table 5. Effect of ethylene and stage of maturity on flour characteristics.

Sample	Dry matter basis				
	Per cent moisture	Per cent protein	Per cent ash	Pigments p.p.m.	Per cent flour extraction
AD	11.5	11.54	0.58	3.89	71.1
AED	11.0	11.04	0.62	3.90	67.0
BD	11.1	10.93	0.48	3.62	69.5
BED	11.4	10.93	0.45	3.58	69.5
BDRD	11.2	11.01	0.49	3.43	70.4
BDRED	12.9	10.47	0.37	3.29	71.0
C	12.8	11.09	0.50	3.29	71.5
CE	12.7	11.10	0.49	3.25	71.3
CRD	12.7	10.48	0.38	3.07	71.3
CRED	12.6	10.97	0.47	3.25	72.1

Table 6. Effect of ethylene on flour and dough characteristics.

Sample	14 per cent moisture basis			
	Per cent absorption by Farinograph	Valorimeter reading	6-hour gassing power mm Hg	Maltose value mg maltose
AD	55.2	28	342.0	195
AED	55.8	22	370.2	254
BD	55.1	27	334.5	182
BED	54.6	30	295.5	165
BDRD	55.5	30	298.5	171
BDRED	51.0	36	242.0	125
C	52.5	32	280.0	147
CE	52.8	32	270.5	140
CRD	51.0	38	226.0	127
CRED	53.2	35	267.5	153

Breadmaking Quality

To determine the breadmaking quality of the flour, samples were baked in duplicate, and repeat of the bake was made a few days later. To determine the approximate mixing time, absorption and bromate response, a composite of the 10 samples was made and baked. The addition of an optimum amount of bromate to the mix causes an optimum development of the dough, other factors being equal. The optimum bromate requirement of a flour is determined from the maximum gain in volume and external and internal appearance of the loaf. In the following bakes a bromate series of 0, 1, 2, 3 mg was used for samples AD and AED. The optimum bromate requirement for sample AD was found to be 2 mg while there was no apparent improvement in sample AED with the addition of bromate. A bromate series of 0, 1, 2 mg was used for the remaining eight samples. Here the optimum bromate requirement was found to be one mg. The optimum mixing time for samples AD and AED determined by baking was one and one-half minutes and one and three-fourths minutes, respectively, for the remaining eight samples. Further baking data are given in Table 7.

Table 7. Effect of ethylene on breadmaking quality.

Sample	Loaf volume in cc				Per cent absorption	Crumb grain			
	Mg $KBrO_3$					Mg $KBrO_3$			
	0	1	2	3		0	1	2	3
AD	650	691	705	717	57.5	74	76	76	77
AED	579	593	580	592	58.0	64	65	65	65
BD	650	715	727	-	58.0	80	82	84	-
BED	666	723	717	-	57.5	81	82	83	-
BDRD	665	732	725	-	58.0	81	82	83	-
BDRED	697	726	725	-	56.5	81	83	84	-
C	710	745	750	-	56.5	84	86	86	-
CE	705	737	771	-	56.5	85	86	87	-
CRD	727	756	747	-	56.5	84	85	86	-
CRED	696	744	757	-	57.0	84	86	87	-

DISCUSSION

The data obtained reveal that a number of interesting changes occurred in the maturing wheat analyzed in these studies which may in some cases have been influenced by both the unusual climatic conditions prevailing during the harvest period as well as by the ethylene treatment.

Factors which were affected by the maturity of the grain may be summarized as follows. The data of Table 3 indicate that mineral matter decreased with an increase in maturity of the sample which agree with the findings of McCalla and Newton (22) and others for normal wheat and which obviously is unaffected by the treatment with ethylene. Table 3 also indicates a decreasing trend in the fat acidity of the wheat, which indicates a synthesis of triglycerides from free fatty acids which is to be expected with maturation of the seed. The data of Table 4 indicate a possible decrease in proteolytic activity and peroxidase activity with maturation of the grain, substantiating the work of Bach et al. (4). Table 4 also shows an increase in germination with an increase in maturity of the wheat as has been found by Atkinson and Jahnke (3) for normal wheat. Table 5 shows a slight decrease in the flour pigments with an increased maturity of the grain from which the flour was milled. This modification also is a normal change with maturity since chlorophyll and other pigments present in

the filling kernel are bleached out with increasing maturity. The gassing power and maltose value of the flour decreases as is shown in Table 6. Since these two factors are partially dependent on the amylase activity, these data substantiate the work of Kretovich (19), who found that amylase activity almost disappeared in the process of ripening of grains. Table 7 indicates that an improvement in breadmaking quality of the flour occurred with the maturation of the grain from which it was milled. This wheat was exposed to several rains while standing in the field as is shown in Table 1. Swanson (31) harvested wheat at different stages of maturity which had been exposed to rain and found that there was an improvement in the baking quality of the wheat harvested at later stages of maturity.

All of the findings listed up to this point are normal changes which are to be expected in a maturing wheat seed. Virtually no influence due to the ethylene treatment is apparent on these very important biochemical indices of maturity.

The data in Table 5 indicate that ethylene increased the nonprotein nitrogen of the wheat. This seemed to occur in all of the samples except that harvested at 12 per cent moisture and rewetted. Table 3 also suggests that ethylene may have produced a decrease in fat acidity of the samples harvested at 38 per cent and 20 per cent moisture. The data of Table 4 indicate that ethylene increased the germination of Samples BD and C, which is in agreement with the findings of Balls and Hale (5), while it seemed to decrease the germination of samples BDRD

and CRD. The data of Table 4 also indicate that ethylene decreased the proteolytic activity of sample AD and increased the proteolytic activity of samples B, BD, C and CRD. Ethylene treatment seemed to decrease the per cent flour extraction of sample AED as is indicated by the data of Table 5.

The data of Table 6 indicate that ethylene treatment appeared to be related to an increase in the gassing power and maltose value of samples AD and CRD, and decreased these two factors in sample BD and BDRD although such variation could be ascribed to the milling treatment as previously indicated. The graphs of Plate I also indicate that ethylene increased the gassing power of samples AD and CRD and decreased it in samples BD and BDRD. The data of Table 7 and the photographs in Plate II indicate that ethylene decreased the breadmaking quality of sample AD. Gas retention primarily, rather than gassing power and maltose value, determine the loaf volume, although there is a correlation between gassing power, maltose value, and loaf volume. The small loaf volumes obtained with sample AED were probably due to a low gas retention, which may in some way have been affected by the ethylene treatment.

The rewetting and drying of samples BD and C increased the germination of the wheat as is indicated by the data in Table 4. Table 6 suggests that the rewetting and drying of samples BD and C decreased the gassing power and maltose value of the flour. This is not in agreement with the work of Swanson (30) who found that rewetting the wheat in the shock caused an in-

crease in the maltose value of the flour. Drying of the damp grain, either natural or rewetted, showed no apparent effect on the wheat. There seemed to be no special effect of ethylene when applied after the rewetting treatment.

Previous studies of the effects of ethylene on freshly-harvested wheat have been very incomplete. In unpublished experiments, Hale (15) found that the ethylene treatment of a commercial lot of freshly-harvested wheat appeared to increase the ease of milling. This is of particular commercial significance. Any practical method of treatment which would condition wheat so that it would have similar tempering requirements throughout the crop season, mill more uniformly, and produce flour which requires uniformly constant oxidation, would be of great practical value to the milling industry.

Any great usefulness of ethylene treatment of wheat immediately after harvest to improve the milling and baking properties of fresh wheat did not appear from the present studies. There is no doubt that it has been shown to have a profound effect on hastening the maturation of certain fruits and vegetables, and that its use has proved of great economic value to those industries. The work of Balls and Hale (5), and Hale, Schwimmer and Bayfield (16) who found that not only was the keeping quality of wheat greatly improved by this treatment but also that the baking quality of the flour was definitely bettered, cannot be taken lightly even though the data obtained

EXPLANATION OF PLATE I

The effect of ethylene on the gassing power of flour samples milled from freshly-harvested wheat. The gassing power of the ethylene-treated sample is represented by curve b and the untreated by curve a.

Fig. 1. Samples AD and AED

Fig. 2. Samples BD and BED

Fig. 3. Samples BDRD and BDRED

Fig. 4. Samples C and CE

Fig. 5. Samples CRD and CRED

PLATE I

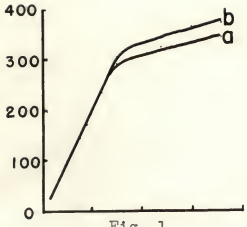

Fig. 1.

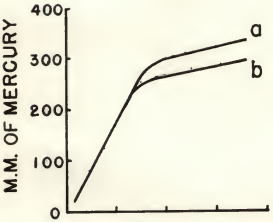

Fig. 2.

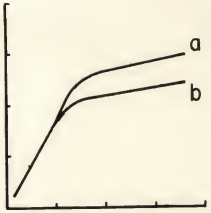

Fig. 3.

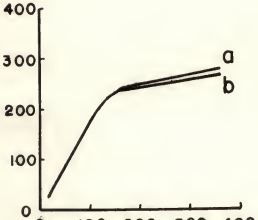

Fig. 4.

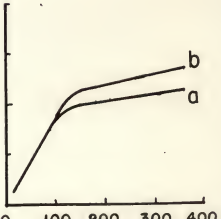

Fig. 5.

TIME IN MINUTES

EXPLANATION OF PLATE II

The effect of ethylene on the baking properties of flour milled from freshly-harvested wheat. Pictures show the bromate response of the flour.

Fig. 6. Sample AD

Fig. 7. Sample AED

Fig. 8. Sample AD

Fig. 9. Sample AED

Loaf No. 1. 0 mg bromate

Loaf No. 2. 1 mg bromate

Loaf No. 3. 2 mg bromate

Loaf No. 4. 3 mg bromate

Loaf No. 5. 0 mg bromate

Loaf No. 6. 1 mg bromate

Loaf No. 7. 2 mg bromate

Loaf No. 8. 3 mg bromate

PLATE II

Fig. 6.

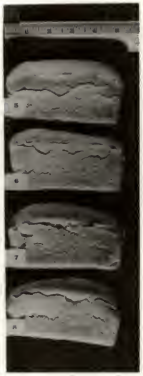

Fig. 7.

Fig. 8.

Fig. 9.

in the present study failed to confirm the results of these previous studies. Other factors are doubtless responsible for the lack of agreement.

A major factor in the present investigation was the abnormal maturing season in which recurrent rains caused a fluctuation of the moisture content in the immature grain before it was in condition for harvesting (Table 1). It seems reasonable to suppose that once the vegetative phase of filling of the grain is completed, the desiccation phase which normally follows not only results in a loss of moisture but also in a change of active constituents, such as enzymes, into inactive forms. This has been shown for seeds by Bach, Oparin and Vener (4). It would seem possible that one of the influences of ethylene on plant metabolism is a speeding up of conversions of this sort which normally occur slowly in the maturation phase. One must nevertheless assume that the prolonged time in which the crop stood during damp weather after filling was complete and desiccation was inhibited, allowed these physiological changes to continue to completion before the ethylene treatment was applied. The excellent baking response of the flours produced from these wheats in view of their low protein content and the low oxidation requirement for maximum loaf volume certainly suggests that this prolonged period of standing in damp weather before harvest did no harm to the baking properties and indeed apparently caused a profound improvement (Plates III and IV). These results are clearly in line

with the studies of Swanson (31) on the effect of rains during harvest on wheat quality.

It is hoped that the results of this study will not discourage but rather stimulate further study on the problem of ethylene treatment of freshly harvested wheat. Another year when the weather conditions during the harvest season are more favorable may produce results more consistent with those previously reported by other workers.

EXPLANATION OF PLATE III

The effect of ethylene on the baking properties of flour milled from freshly-harvested wheat. Pictures show the bromate response of the flour.

Figs. 10 and 14. Sample BD
Figs. 11 and 15. Sample BED
Figs. 12 and 16. Sample BDRD
Figs. 13 and 17. Sample BDRED

Loaf No. 9, 12, 15 and 18. 0 mg bromate
Loaf No. 10, 13, 16 and 19. 1 mg bromate
Loaf No. 11, 14, 17 and 20. 2 mg bromate

PLATE III

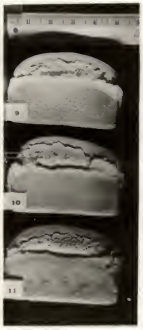

Fig. 10.

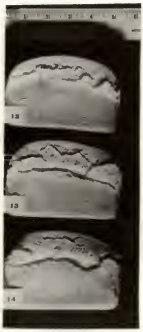

Fig. 11.

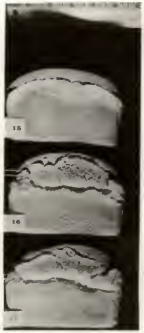

Fig. 12.

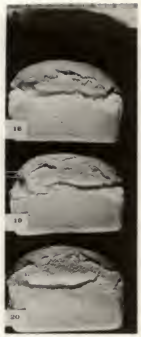

Fig. 13.

Fig. 14.

Fig. 16.

Fig. 15.

Fig. 17.

EXPLANATION OF PLATE IV

The effect of ethylene on the baking properties of flour milled from freshly-harvested wheat. Pictures show the bromate response of the flour.

Figs. 18 and 22. Sample C
Figs. 19 and 23. Sample CE
Figs. 20 and 24. Sample CRD
Figs. 21 and 25. Sample CRED

Loaf No. 21, 24, 27 and 30. 0 mg bromate
Loaf No. 22, 25, 28 and 31. 1 mg bromate
Loaf No. 23, 26, 29 and 32. 2 mg bromate

PLATE IV

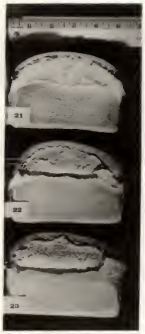

Fig. 18.

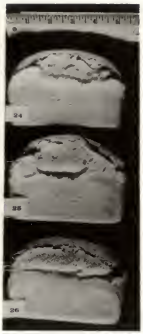

Fig. 19.

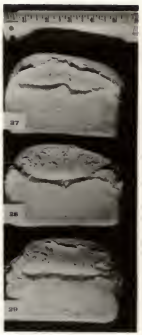

Fig. 20.

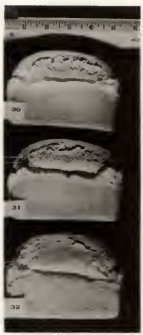

Fig. 21.

Fig. 22.

Fig. 24.

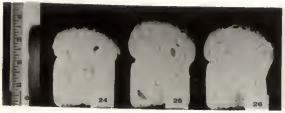

Fig. 23.

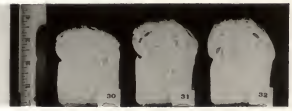

Fig. 25.

SUMMARY

Since it is believed that ethylene accelerates the metabolic and life processes of a seed, it seemed worthwhile to study the effect of this gas on the biochemical and breadmaking properties of freshly-harvested wheat. The chemical and biochemical properties, including milling and baking quality of wheat harvested at various stages of maturity, were studied before and after treating the wheat with ethylene. A major factor in this study, which makes it almost impossible to draw decisive conclusions, was the abnormal maturing season in which recurrent rains required the grain to remain in the field for a longer than normal period and caused a fluctuation of the moisture content in the immature grain before it was in condition for harvesting. The results of this study demonstrate that:

1. There was a decrease in mineral matter, fat acidity, proteolytic activity, and peroxidase activity with maturation of the wheat.

2. There was an increase in germination of the wheat with increasing maturity of the grain.

3. A decrease in pigments, maltose value and gassing power of the flour with maturation of the wheat from which it was milled, occurred.

4. An improvement resulted in the breadmaking quality of

the flour with maturation of the grain from which it was produced. It is possible that this improvement may have been due to exposure to rains in the field.

5. Ethylene increased the nonprotein nitrogen content of the wheat.

6. Ethylene produced a decrease in fat acidity of the wheat harvested at 38 per cent and 20 per cent moisture.

7. Ethylene increased the germination of the wheat harvested at 20 per cent and 12 per cent moisture and decreased the germination when treatment was applied after rewetting the dried wheat.

8. Ethylene decreased the proteolytic activity of the wheat harvested at 38 per cent moisture and increased the proteolytic activity of the wheat harvested at 20 per cent and 12 per cent moisture.

9. Ethylene decreased the per cent flour extraction of the wheat harvested at 38 per cent moisture and did not increase the ease of milling of any of the wheat samples.

10. Ethylene appeared to increase the gassing power and maltose value of the flour milled from the dried wheat which was harvested at 38 per cent moisture and the rewetted and dried wheat which was harvested at 12 per cent moisture. The ethylene also decreased the gassing power and maltose value of the flour milled from the dried and rewetted and dried wheat which was harvested at 20 per cent moisture. Milling treatment of these samples might also account for those variations to some

extent.

11. Ethylene decreased the breadmaking quality of the flour milled from the wheat harvested at 38 per cent moisture.

12. Treatment with ethylene caused no worthwhile improvement in the milling and baking properties of the wheat investigated in this study.

ACKNOWLEDGMENTS

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APPENDIX

In order to determine any difference in the course of natural aging of wheat due to treatment of the freshly harvested grain with ethylene, the remainder of the wheat samples were milled and baked after approximately one month of storage at about 30° C. The wheat samples were milled on August 24, 1948. Flour extraction data are given in Table 8. These flour samples were baked on September 2, 1948 together with the flour samples BD, BED, C and CE which were milled by General Mills, Inc. from this same wheat. The baking formula and procedure used were identical to those previously employed. The per cent protein and ash of the flours milled at Kansas State College also are given in Table 8. The loaf volume data presented in Table 8 show an improvement only in sample AED which is attributable to the aging in storage. The data on the flour samples milled by General Mills are given in Table 9, and indicate that a lower extraction was obtained in the milling of these samples since the protein and ash contents are both lower than those milled at Kansas State College. The loaf volumes given by these flours were moderately superior to those given by the same flours milled here in the case of the "B" series but somewhat less than those obtained with the "C" series milled in this laboratory.

Table 8. Milling and baking properties of flour samples milled from wheat stored for one month. Samples milled at Kansas State College.

Sample	Per cent moisture	Dry matter basis: Per cent protein	Per cent ash	Mg KBrO3	Loaf volume cc	Flour extraction 14% M.B.
AD	13.5	11.2	0.50	2	727	58.5
AED	13.0	11.3	0.59	2	647	67.0
BD	13.5	11.1	0.49	1	732	67.5
BED	13.5	11.2	0.46	1	735	67.5
BDRD	13.3	11.1	0.46	1	715	70.5
BDRED	13.3	11.1	0.47	1	727	70.0
C	13.5	11.0	0.46	1	735	68.5
CE	13.2	11.1	0.46	1	742	69.5
CRD	13.1	11.3	0.46	1	747	69.0
CRED	12.9	11.3	0.46	1	732	68.0

Table 9. Milling and baking properties of flour samples milled by General Mills, Inc.

Sample	Per cent moisture	Dry matter basis: Per cent protein	Dry matter basis: Per cent ash	Mg $KBrO_3$	Loaf volume cc 14% M.B.
BD	11.6	10.6	0.37	1	740
BED	11.7	10.8	0.38	1	755
C	11.6	10.6	0.37	1	695
CE	11.7	10.6	0.36	1	697